Day 1

To begin our lab work, we performed nine different transformations following the iGEM transformation protocol. Nine different plasmids were introduced into competent *E. coli* cells. Each *E. coli* sample was then plated (100 µl) and incubated overnight. Three different concentrations of RFP were used as a control (5,10,20).
Samples Included: B0015, J23118,C0040, B0034,J23100,J23114,J33201, J45014, and K523013
Outside of the lab we discussed our overall project goals as well as outreach programs including a "women in science" day with a synthetic biology presentation.

Day 2

Following up on our transformations from the first day we performed cell cultures of the nine transformed samples. We also made our own stock of LB Liquid Medium as well as antibiotic plates including LB+KAN, LB+AMP, and LB+CAM. Further, we attended a department wide biological safety seminar, and performed gel electrophoresis to practice the technique and learn where the materials are in lab. Further, it allowed us the practice reading the ladder.

Day 3

During our third day in the lab we made a glycerol stock to preserve our original plasmids. Then, we purified our plasmid samples by performing minipreps according to the iGEM protocol. Using a nanodrop we analyzed the density of our plasmid samples. In the afternoon the team split up and began working on separate projects.

- Shawna and Corbyn began working with BCLA and CAEV-p28. We performed the transformations of 10A and 11K according to the iGEM protocol. We then plated the *E. coli* transformed with 10A and diluted (1:10) 10A on LB+AMP plates and the *E. coli* transformed with 11k and the diluted (1:10) 11k *E. coli* on LB+KAN plates. These were incubated at 37 degrees overnight. As a negative control the undiluted 10A *E. coli* were plated on an LB+CAM plate to test the strength of the antibiotic on the plate. The CAM plates were being tested because the p1000 pipette used to prepare the LB media was found to be poorly calibrated. We also prepared a cell culture of the 9C *E. coli* in 5mL of SOC with 5 microliters of chloramphenicol. This was incubated at 37 degrees over night in a shaker.
    - **Results:** See pictures on google drive, undiluted created lawns and 1:10 dilution created a few single colonies, in the future we ***recommend*** more than a 1:10 dilution
- Alex and Chloe ordered YFP oligos, confirmed INP-YFP expression and membrane integration, and transformed YFP plasmids (BBa_E0030) according to the iGEM protocol.
    - **Results:**
- Kayla and Mike started to construct the banana odor arsenic detector. Restriction enzymes were used to cut the arsenic promoter (BBa_J33201) and the ribosome binding site (BBa-B0034) out of their respective plasmids as well as to cut the

linearized kanamycin resistant plasmid (psB1K3). The iGEM restriction digest protocol was followed.

iGEM Day 4

A protocol was proposed for the agglutination assay. Each team performed the agglutination assay according to the initial protocol proposal.

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- Kayla and Mike followed the iGEM ligation protocol for linearized plasmids to ligate the arsenic promoter and the ribosome binding site into the kanamycin resistant linearized plasmid. Following the ligation, competent *E. coli* cells were transformed using the newly constructed plasmid and plated on kanamycin plates. The plates were incubated overnight.

- Shawna and Corbyn worked on designing and testing a laboratory activity for the Women in Science camp to perform during their upcoming visit. The team decided upon an experiment involving running food coloring through gel electrophoresis. The detailed procedure was outlined after the experiment was tested and a final procedure was decided upon (it can be found in the procedure folder). In the afternoon, cell cultures were made from the 10A and 11K transformations that were plated the previous day. These were incubated in the 37 degree shaker overnight. When performing the agglutination protocol, we had the monoclonal GFP antibody and used BSA as our buffer. Results can be seen in the photo folder for week 1.

Day 5

- Kayla and Mike started off the day by checking the transformation plate. No bacteria grew, so the plasmid ligation did not proceed as expected. Thus, the arsenic promoter, the ribosome binding site, and the kanamycin resistant plasmid were re-digested using four times the mass of the arsenic promoter and ribosome binding site plasmids and then re-ligated in attempt to obtain the proper ligation product. To assure that the plasmids containing the ribosome binding site and the arsenic promoter were being properly digested by the restriction enzymes, the digests were run on a gel. For the arsenic promoter, a band for the linearized plasmid and a band for the arsenic promoter piece appeared on the gel as expected. For the ribosome binding site, only a band for the linearized plasmid appeared because the ribosome binding site sequence is so short that the band traveled beyond the end of the gel.
- Chloe prepared a miniprep of 12C cell cultures, brought the 9C culture from Day 4 up to 25 mL and incubated it at 37 degrees for 5 hours until it was 0.9 OD for use in the second agglutination test trial, performed a PCR on 12C DNA, and ran PCR product on a gel (Success, results can be found in week 1 photo folder)
- Shawna and Corbyn created a glycerol stock of 10A and 11K to preserve the original plasmids. Then, we purified the 10A and 11K plasmid samples by performing minipreps according to the iGEM protocol. Then, using a nanodrop we analyzed the density of our plasmid samples. We also edited both the rainbow gel protocol as well as the

agglutination protocol. Corbyn and Shawna also digested 10A and 11K following iGEM protocl using various enzyme to create biobricks and to begin our parts assembly of a backbone with both BCLA and CAEV.